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FURTHER OBSERVATIONS ON THE SPECIFICITY OF ANTIGEN 5 OF *ECHINOCOCCUS GRANULOSUS*

Luis A. Yarzabal, Daniel T. Bout, Frida R. Naquira, and André R. Capron
Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, 20, boulevard Louis XIV, 59012 LILLE, France

ABSTRACT: The presence of IgE antibodies to antigen 5 of *Echinococcus granulosus* was detected by means of radioimmunoelectrophoresis in the sera of two of six patients infected with *E. multilocularis*. Sera from three of these patients gave a precipitin band in gel diffusion tests identical to that produced by a monospecific rabbit anti-*E. granulosus* antigen 5 serum, when tested against whole hydatid fluid. Sera from 19 individuals infected with *Fasciola hepatica*, 20 with *Schistosoma mansoni*, and 5 with with *Taenia saginata* showed no detectable antibodies against antigen 5 of *E. granulosus*. The monospecific rabbit anti-*E. granulosus* antigen 5 serum did not react in immunodiffusion with homologous antigen when absorbed with either 4 mg/ml of whole hydatid fluid or with 200 mg/ml of a soluble *E. multilocularis* extract. Absorption of the monospecific antiserum with crude antigens of either *F. hepatica*, *Onchocerca volvulus*, *S. mansoni*, or *T. saginata* did not abolish the reaction with antigen 5. It appears, therefore, that antigen 5 can no longer be considered specific for *E. granulosus*, but is also present in *E. multilocularis*. In the light of this observation, some reevaluation of immunodiagnostic tests in hydatid disease will be necessary.

INTRODUCTION

The adaptation of radioimmunoelectrophoresis to the study of IgE antibodies in helminth infections (Bout et al., 1977) has permitted the identification of several different allergens in hydatid cyst fluid. Antigen 5 (Capron et al., 1967) and antigen B (Oriol et al., 1971) have been shown to be distinct and until recently were considered specific for *E. granulosus*. However, IgE antibodies interacting specifically with antigen 5 of *E. granulosus* were detected by Bout et al. (1977) in serum of a patient with multilocular echinococcosis. This suggests that antigen 5 of *E. granulosus* or some of its determinants, forms part of the antigenic mosaic of *E. multilocularis*. Since immunodiagnostic tests are presently interpreted considering that arc 5 is highly specific for *E. granulosus*, we have attempted to clarify the situation in the following study. Sera from patients with multilocular echinococcosis were examined by radioimmunoelectrophoresis for the presence of IgE antibodies to antigen 5. Immunoprecipitation and immunoabsorption studies on extracts of *E. multilocularis*, *Taenia saginata*, *Fasciola hepatica*, *Onchocerca volvulus*, and *Schistosoma mansoni* were performed in an attempt to demonstrate the presence of antigen 5.

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MATERIALS AND METHODS

Human sera

Sera were obtained from 12 patients infected with *Echinococcus* (six with *E. granulosus* and six with *E. multilocularis*) confirmed by examination of surgical material; 20 patients infected with *S. mansoni*; 19 patients infected with *F. hepatica*; five patients with taeniasis (*T. saginata*) and six apparently healthy individuals which were included as controls. The diagnosis was confirmed in all cases by previous serological and parasitological examination. Only the sera from individuals infected with *Echinococcus*, and two control groups (*F. hepatica* infected and normal individuals) were examined by radioimmunoelectrophoresis (RIEP). The samples analyzed by RIEP were selected from sera stored at –20 C at the "Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, Lille," on the basis of their high titer of circulating IgE. The titer had been previously determined by Dessaint et al. (1975).

Experimental sera

RaHF was prepared by injecting 40 mg of lyophilized whole hydatid fluid into the axillary regions of an adult rabbit, according to the procedure described by Capron et al. (1967).

Ra5 was prepared following the method of Bout et al. (1974). The precipitin band containing the desired antigen was extracted from bidimensional immunoelectrophoresis slides. After washing for 48 hr in phosphate buffered saline, the agar containing the band was broken up in 0.5 ml of saline and then emulsified in 0.5 ml of Freund's complete adjuvant. This antigen-antibody-adjuvant mixture was injected intradermally into a rabbit according to the procedure of

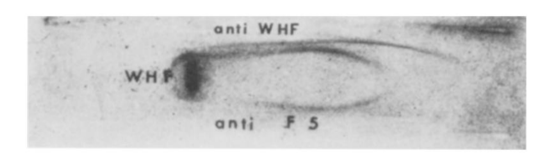


FIGURE 1. Radioimmunoelectrophoresis show- IgE of serum from *E. multilocularis*-infected patient fixed to antigens of whole hydatid fluid, including antigen 5 (Capron et al., 1967). WHF = whole hydatid fluid. Anti-WHF = rabbit immune serum anti-whole hydatid fluid. Anti-F 5 = rabbit immune serum anti-*E. granulosus* antigen 5.

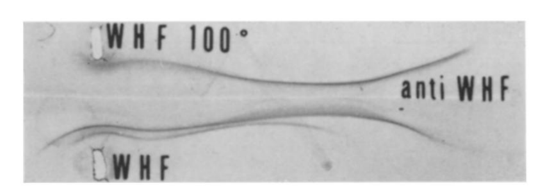


FIGURE 2. Radioimmunoelectrophoresis showing IgE of serum from *E. multilocularis*-infected patient fixed to the thermostable allergen of hydatid fluid: antigen B (Oriol et al., 1971). WHF 100° = boiled whole hydatid fluid. Anti-WHF = rabbit immune serum anti-whole hydatid fluid. WHF= whole hydatid fluid.

Vaitukaitis et al. (1971), and serum was collected 15 days later. The monospecificity of the immune serum was determined by immunodiffusion and immunoelectrophoretic techniques.

^{125}I labeled rabbit anti-human IgE antibodies were purchased from Pharmacia (Uppsala).

Antigens

The soluble antigen fraction from *T. saginata*, *F. hepatica*, *O. volvulus*, and *S. mansoni* were extracted as described by Capron et al. (1968).

The *E. multilocularis* extract was obtained from hepatic cysts of experimentally-infected *Clethrionomys rutilus* (female) experimentally-exposed to *E. multilocularis* from *Alopex lagopus** with the method employed by Capron et al. (1970b). Briefly, the cysts were aseptically removed, homogenized, extracted in NaCl 1‰, centrifuged, dialyzed, and freeze-dried.

Whole hydatid fluid was prepared according to the procedure described by Capron et al. (1967).

Techniques

Radioimmunoelectrophoresis, based upon the procedure described by Yagi et al. (1962), was carried out in four steps: (1) after electrophoresis, the soluble *E. granulosus* antigens were precipitated with either immune sera, anti-whole hydatid fluid or anti-antigen 5; (2) next, the sera from infected patients was applied to the troughs allowing the specific IgE antibodies to bind to any free antigenic determinants in the precipitate; (3) radiolabeled anti-human IgE antibodies were then fixed to the specific anti-parasitic IgE antibodies; and (4) finally the labeled bands were detected by autoradiography.

Unidimensional immunoelectrophoresis (IEP) was carried out as described in Capron et al. (1967). The bidimensional variant was performed according to the procedure of Axelsen (1971). The procedure for immunodiffusion was a slight variation of the techniques of Abelev (1960) and Ouchterlony (1948).

* This material was kindly supplied by Doctor R. Rausch.

In the immunoprecipitation inhibition test the monospecific rabbit anti-antigen 5 serum was distributed in aliquots of 25 μl in 6 series of 14 tubes each. A different antigen was added in concentrations varying from 4 to 200 mg/ml to each series. The resultant mixtures were incubated for 2 hr at 37 C and then for 16 hr at 4 C. Afterwards each was centrifuged at 2,500 g. The absorbed serum was tested by immunodiffusion against whole hydatid fluid containing antigen 5, and also against the antigen used in the absorption. For this test each antigen was used at a concentration of 100 mg/ml.

RESULTS

Reaginic antibodies in sera from hydatid patients with a high IgE titer reacted with a wide range of whole hydatid fluid antigens. In all cases the precipitant system corresponding to antigen 5 was revealed by autoradiography.

Two serum samples from patients infected with *E. multilocularis* contained IgE type antibodies which interacted with antigens 5 (Capron et al., 1967) and B (Oriol et al., 1971) of whole hydatid fluid (Figs. 1, 2).

Sera from control groups (fasciolasis patients and healthy donors) did not react with any of the hydatid antigens when assayed by radioimmunoelectrophoresis.

When assayed by immunodiffusion and IEP using whole hydatid fluid, three of the sera (concentrated 6×) from patients infected with *E. multilocularis* gave a precipitation line identical to that formed with human and rabbit anti-antigen 5 sera (Fig. 3).

However, serum samples from 20 individuals infected with *S. mansoni*, 19 from patients suffering from *F. hepatica* infections, and five from individuals infected with *T. saginata*

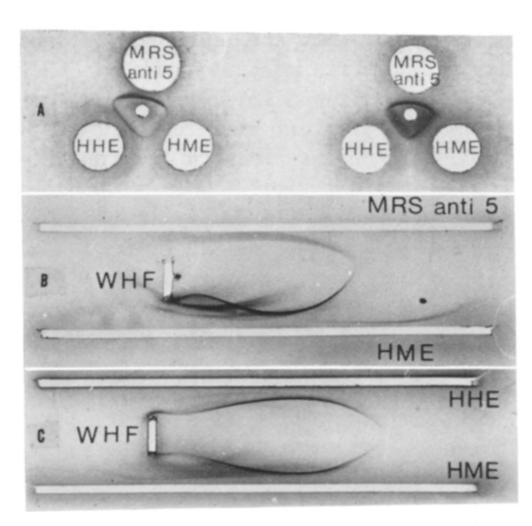


FIGURE 3. Immunoprecipitation tests showing immunological identity between the precipitant system formed by rabbit monospecific anti-antigen 5 and one of the precipitant systems formed by sera from hydatid and multilocular patients against WHF. (A: double diffusion test, B and C: immunoelectrophoresis test). MRS anti-5 = rabbit monospecific anti-antigen 5. HHE = human hydatid echinococcosis serum. HME = human multilocular echinococcosis serum. WHF = whole hydatid fluid.

failed to react with antigen 5 when assayed similarly.

Absorption of the specific antisera with lyophilized whole hydatid fluid at a concentration of only 4 mg/ml would inhibit the immunodiffusion test. Inhibition was not obtained with crude extracts of *T. saginata*, *F. hepatica*, or *S. mansoni* even with a concentration exceeding 200 mg/ml.

Substantial loss in activity could be achieved in sera absorbed with extracts of *E. multilocularis* at a concentration of 20 mg/ml. Total inhibition was produced at 200 mg/ml.

Monospecific rabbit anti-antigen 5 serum failed to reveal any antigenic components in extracts of *E. multilocularis*, *T. saginata*, *F. hepatica*, *O. volvulus*, or *S. mansoni* when assayed by immunodiffusion and immunoelectrophoresis.

DISCUSSION

Results presented here indicate that antigen 5 of *E. granulosus* is also a component of soluble extracts of *E. multilocularis*. Antigen 5 was originally identified by Capron et al. (1967) in horse hydatid cyst fluid when assayed by immunoelectrophoresis. The substance appears to be accumulated at high concentration and results extremely immunogenic. By the use of homologous and heterologous hyperimmune sera in immunoprecipitation studies, Capron et al. (1967) failed to reveal antigen 5 in *E. multilocularis* soluble extracts. They also failed to demonstrate the presence of the product in soluble extracts of other parasitic helminths (*Taenia*, *Moniezia*, *Anoplocephala*, *Fasciola*, *Dicrocoelium*, *Paragonimus*, *Schistosoma*, *Ascaris*, and *Dipetalonema*), suggesting that it is a species-specific antigen.

In further studies, the antigen was identified in hydatid fluid from bovine (Yarzabal et al., 1974) and ovine (Varela-Diaz et al., 1974). It was purified and partially characterized by Bout et al. (1974). These authors showed that it is a thermolabile lipoproteic substance which supports alpha and beta carboxylesterase activities. They estimated its molecular weight to be approximately 60,000 daltons.

Analysis of the immunoelectrophoresis diagrams and immunodiagnostic results presented by Chordi and Kagan (1965) suggests that antigen 5 corresponds to the antigen 4 described by these authors. This antigen, recently isolated by Pozzuoli et al. (1975), proved to be the most immunoreactive parasitic antigen of sheep hydatid fluid, and showed a high degree of specificity. The possible immunodiagnostic importance of antigen 5 was emphasized by Capron et al. (1967) who revealed the presence of precipitating antibodies to antigen 5 in sera from 21 confirmed hydatidosis cases. Subsequent studies (Capron et al., 1970b; Quilici et al., 1971; Yarzabal and Capron, 1971; Yarzabal et al., 1974; Varela-Diaz et al., 1975) indicated that sera from a high percentage of surgically proven hydatid patients had circulating antibodies to antigen 5. However, with the exception of the work of Capron et al., 1970b, these studies did not examine sera from patients infected with *E. multilocularis*.

On the basis of these results it is generally accepted that the identification of antibodies to antigen 5 in sera from symptomatic or

asymptomatic human cases, is a solid argument for the diagnosis of *E. granulosus* infection.

The discrepancies between present findings and those of Capron et al. (1970 a, b) may be explained by the different techniques used in each study. In previous experiments, 1 ml of the anti-whole hydatid fluid antiserum (containing anti-antigen 5 antibodies) had been absorbed with 30 mg of *E. multilocularis* extract. In our procedure, we mixed increasing amounts of *E. multilocularis* antigen with a constant amount of monospecific anti-5 immune serum, achieving a complete absorption of anti-5 antibodies with a concentration 50 folds higher of the heterologous extract.

The necessity to use this amount of *E. multilocularis* antigen to inhibit the immunoprecipitation of the monospecific rabbit antiserum-whole hydatid fluid system, suggest that the concentration of antigen 5 is weak in *E. multilocularis* extract. This may explain the results published by Farag et al. (1975). They used the antigen purified by Bout et al. (1974) in an attempt to assess the feasibility of diagnosis of hydatid disease by means of the enzyme-linked-immunosorbent-assay (ELISA), and showed statistically significant differences in the reactivity of their groups of hydatid and multilocular sera. They claimed that these observations made the differential diagnosis of the two major forms of human echinococcosis possible, but their results showed a slight interaction between antigen 5 and multilocular echinococcosis sera.

The absence of any detectable interaction between circulating antibodies from *E. multilocularis* infected patients and *E. granulosus* antigen 5 is a more perplexing problem (Capron et al., 1970a, b). Possibly, the number of multilocular echinococcosis cases examined in earlier studies was insufficient and failed to include any anti-antigen 5 reacting sera. Alternatively qualitative or quantitative variations in antigenic composition of different strains of *E. multilocularis* may exist. This hypothesis is supported by the work of Smyth and Davies (1975) who observed metabolic differences between horse and sheep strains of *E. granulosus*. This may explain the absence of anti-antigen 5 antibodies in a number of *E. multilocularis* infected patients because of the lack of this antigen as a metabolic product of the particular strain.

In conclusion, our results provide new information on the antigenic relationship between *E. granulosus* and *E. multilocularis*. The implications of our findings on immunodiagnosis of hydatid disease are nevertheless limited and affect only the diagnostic value of specific band 5 in areas where *E. granulosus* and *E. multilocularis* may coexist. Identification of band 5 can no longer be used to differentiate hydatid disease from *E. multilocularis* infections.

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